

Assessment of mitochondrial toxicity of environmental chemicals using a quantitative high throughput screening approach

M.S. Attene-Ramos¹, R. Huang¹, S. Sakamuru¹, S. Shahane¹, L. Shou¹, K.L. Witt², R.R. Tice², C.P. Austin² and M. Xia¹

¹National Institutes of Health Chemical Genomics Center (NCGC), Bethesda, MD 20892,

²National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709



Abstract

As part of the U.S. Tox21 initiative, the NCGC is developing and optimizing cell-based and biochemical assays suitable for quantitative high throughput screening (qHTS) in a 1536-well format. This effort will generate pathway profiles for environmental compounds that will facilitate the evaluation of mechanisms of toxicity and prioritization for more extensive testing, as well as the development of predictive models for in vivo toxicity. In this study, we optimized a mitochondrial membrane potential assay using the water-soluble JC-10 sensor to detect mitochondrial depolarization in HepG2 cells and we then used this method to evaluate the mitochondrial toxicity of 1408 environmental compounds provided by the NTP. In response to mitochondrial depolarization, the ratio of the cytosolic green fluorescent monomeric form to the mitochondrial red fluorescent aggregate form increases. Of the 1408 compounds screened over a 14-point concentration curve (0.59 nM to 92 μ M), 42 compounds disrupted the mitochondrial potential in HepG2 cells after treatment for one hour. We selected 33 compounds for further studies, including high-content imaging. Thirty-two compounds were confirmed in both fluorescence plate reader and imaging assay formats. To study the structure-activity relationship of these mitochondrial disruptors, we clustered these compounds by structural similarity. This analysis resulted in four structural clusters and 15 singletons. These clusters may be useful for identifying structural features associated with mitochondrial toxicity. Our results confirm the robustness of this assay for identifying mitochondrial membrane-potential disruptors in qHTS format. Supported by NIEHS Interagency Agreement Y2-ES-7020-01.

Mitochondrial membrane potential assay

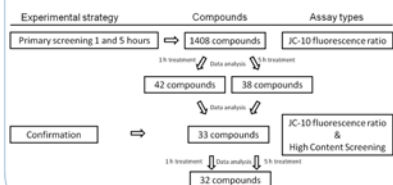
Assay principle: In healthy energized cells, JC-10 accumulates in the mitochondria as red fluorescent aggregates. As the MMP depolarizes JC-10 aggregates are converted to green fluorescent monomers and subsequently released from mitochondria to cytosol.



Primary screening experimental protocol:

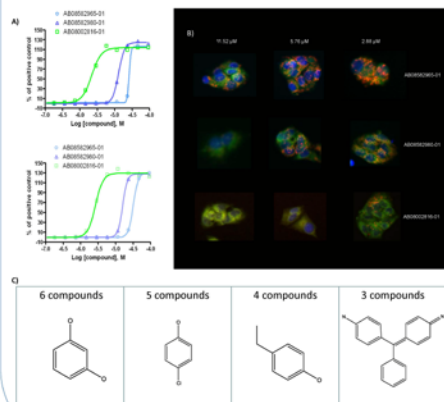
Step	Action	Description
1	Plate cells	2000 HepG2 cells/ well in 5 μ l
2	Incubation	Overnight
3	Library and control compounds	23 nl (0.59 nM to 92 mM, final concentration)
4	Incubation	1 or 5 h
5	Reagent	1 μ l (JC-10 dye)
6	Incubation	30 min, 37° C
7	Assay readout	EnVision Multilabel Reader

Flowchart of identification of mitochondrial toxicants: After the primary screening against NTP 1408 compounds, 42 positives were identified after 1 hour treatment and 38 after 5 hours treatment. Thirty two of 33 compounds positive in both treatment times purchased for the follow-up studies were confirmed in both fluorescence reader based and imaging methods.



Results

A) Example dose response curves for 3 positive compounds after 1 (top) and 5 (bottom) hours treatments. **B)** Images of these 3 compounds at different concentrations. **C)** Common scaffolds yielded from a clustering analysis of positive compounds by structure.



Conclusions

- Of the 1408 environmental compounds tested 32 were confirmed to disrupt the mitochondrial membrane potential using both plate reader and imaging approaches with EC_{50} ranging from 1.6 to 71 μ M.
- Clustering analysis classified the positive compounds into 4 clusters and 15 singletons.
- The use of the JC-10 dye to screen for changes in mitochondrial membrane potential constitutes a robust assay that can be used to screen large compound collections.

References

- Reers M, Smith TW, Chen LB. Biochemistry. 1991; 30: 4480-4486.
- Salvioi S, Ardizzone A, Franceschi C, Cossarizza A. FEBS Lett. 1997; 411: 77-82.